

# Claims

What is claimed is:

- 5                   1.       A method of isolating cells comprising,  
                  (a) obtaining a tissue sample from a subject,  
                  (b) successively exposing the tissue to a first solution with decreasing  
amounts of  $\text{CaCl}_2$  comprising NaCl, HEPES,  $\text{MgCl}_2$ , KCl, and sugar at a pH of  
approximately 7.4,  
10               (c) disassociating the tissue with an enzyme solution,  
                  (d) repeatedly resuspending the disassociated tissue into a second  
solution with increasing amounts of  $\text{CaCl}_2$  comprising Earle's modified salt, L-  
glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid,  
HEPES, fetal bovine serum, an antibiotic, and a fatty acid, at a pH of approximately 7.4  
15       to obtain isolated cells.  
  
                  2.       The method of claim 1, further comprising the step of re-  
suspending the isolated cells approximately every 24 hours in a solution comprising  
Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine,  
20       taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid acid, and  
 $\text{CaCl}_2$  at a pH of approximately 7.4.  
  
                  3.       The method of claim 1, further comprising the step of incubating  
the isolated cells in a mixture of carbon dioxide and air.  
25                   4.       The method of claim 3, wherein the isolated cells are incubated at  
approximately 37°C.  
  
                  5.       The method of claim 1 wherein, the first solution is exposed to the  
30       tissue at approximately 37°C and at approximately 4 ml/min for 3 minutes.  
  
                  6.       The method of claim 1 wherein the concentration of  $\text{CaCl}_2$  in the  
first solution decreases.  
  
35                   7.       The method of claim 1 wherein the first solution comprises  
approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM  
 $\text{MgCl}_2$ , approximately 5.4 mM KCl, and approximately 10 mM D-glucose.

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8. The method of claim 1 wherein the enzyme solution comprises a digestive enzyme.

9. The method of claim 8, wherein the digestive enzyme is a protease or a collagenase.

10. The method of claim 1 wherein the concentration of  $\text{CaCl}_2$  in the second solution increases.

11. The method of claim 1 wherein the enzyme solution comprises approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM  $\text{MgCl}_2$ , approximately 5.4 mM KCl, and approximately 10 mM D-glucose.

12. The method of claim 1 wherein the second solution comprises Earle's modified salt, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, Ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, a fatty acid at approximately 1  $\mu\text{M}$  at a pH of approximately 7.4.

13. A method of isolating cells comprising,  
(a) obtaining a tissue sample from a subject,  
(b) successively exposing at approximately 37°C the tissue to a first solution with decreasing amounts of  $\text{CaCl}_2$  comprising approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM  $\text{MgCl}_2$ , approximately 5.4 mM KCl, and approximately 10 mM sugar at a pH of approximately 7.4,  
(c) disassociating the tissue with an enzyme solution for approximately 8 minutes comprising approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM  $\text{MgCl}_2$ , approximately 5.4 mM KCl, and approximately 10 mM sugar, to form disassociated cells,

(d) repeatedly resuspending the disassociated cells into a second solution with increasing amounts of  $\text{CaCl}_2$  comprising Earle's modified salt, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at approximately 1  $\mu\text{M}$  at a pH of approximately 7.4 to form a solution of isolated cells,

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(e) incubating the isolated cells in a mixture of carbon dioxide and air at approximately 37°C, and

(f) re-suspending the isolated cells approximately every 24 hours in a solution comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and  $\text{CaCl}_2$  at a pH of approximately 7.4 to obtain isolated cells .

14. A method of cultivating isolated cells comprising, resuspending the isolated cells approximately every 24 hours in a solution comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and  $\text{CaCl}_2$  at a pH of approximately 7.4.

15. The method of claim 14 wherein the solution comprises sodium bicarbonate at approximately 1250mg/l, creatine at approximately 328 mg/500ml, taurine at approximately 312 mg/500ml, ascorbic acid at approximately 8.8 mg/500 ml, HEPES at approximately 2.383 g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at approximately 1  $\mu\text{M}$ , and approximately 1mM  $\text{CaCl}_2$ .

16. A cell culture media for cells comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and  $\text{CaCl}_2$  at a pH of approximately 7.4.

17. The cell culture media of claim 16 wherein the media comprises sodium bicarbonate at approximately 1250mg/l, creatine at approximately 328 mg/500ml, taurine at approximately 312 mg/500ml, ascorbic acid at approximately 8.8 mg/500 ml, HEPES at approximately 2.383 g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, a fatty acid at approximately 1  $\mu\text{M}$ , and approximately 1mM  $\text{CaCl}_2$ .

18. A method of isolating cells comprising,  
(a) obtaining a tissue sample comprising cells from a subject ;  
(b) chopping the tissue;  
(c) incubating the tissue in a first solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and nitrilotriacetic acid;

- (d) incubating the tissue in a second solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and a digestive enzyme;
- (e) incubating the tissue in a third solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and a digestive enzyme; and
- (f) centrifuging the tissue to obtain isolated cells .

19. The method of claim 18, further comprising the step of resuspending the isolated cells in a culture media comprising medium M199, BSA, ascorbic acid, taurine, carnitine, creatinine, insulin, and an antibiotic .

20. The method of claim 19, wherein the culture media further comprises a fatty acid or magnesium.

21. The method of claim 18, wherein the first solution comprises approximately 1-2  $\mu\text{M}$   $\text{CaCl}_2$ , approximately 120mM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM  $\text{MgSO}_4$ , approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96.

22. The method of claim 18, wherein the second solution comprises approximately 1-2  $\mu\text{M}$   $\text{CaCl}_2$ , approximately 30  $\mu\text{M}$  NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM  $\text{MgSO}_4$ , approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme.

23. The method of claim 18, wherein the third solution comprises approximately 1-2  $\mu\text{M}$   $\text{CaCl}_2$ , approximately 30  $\mu\text{M}$  NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM  $\text{MgSO}_4$ , approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme.

24. A method of isolating cells comprising,

(a) obtaining a tissue sample comprising cells from a subject ;

(b) chopping the tissue;

(c) incubating the tissue in a first solution comprising approximately 1-2  $\mu\text{M}$   $\text{CaCl}_2$ , approximately 120mM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM  $\text{MgSO}_4$ , approximately 5 mM pyruvate, approximately 20 mM glucose 20,

approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96;

(d) shaking the tissue at approximately 37°C for approximately 12 minutes;

5 (e) bubbling approximately 100% O<sub>2</sub> through the solution;

(f) incubating the tissue in a second solution comprising approximately 1-2 μM CaCl<sub>2</sub>, approximately 30 μM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme;

10 (g) incubating the solution in a third solution comprising third solution comprises approximately 1-2 μM CaCl<sub>2</sub>, approximately 30 μM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme; and

(h) centrifuging the tissue to obtain isolated cells.

25. A method of isolating and cultivating human myocardial cells comprising,

20 (a) obtaining a tissue sample comprising myocardial cells from a human subject;

(b) chopping the tissue;

(c) incubating the tissue in a first solution comprising approximately 1-2 μM calcium, approximately 120mM NaCl, approximately 5.4 mM KCl, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96;

(d) shaking the tissue at approximately 37°C for approximately 12 minutes;

30 (e) bubbling approximately 100% O<sub>2</sub> through the solution;

(f) incubating the tissue in a second solution comprising approximately 1-2 μM, approximately 30 μM NaCl, approximately 5.4 mM KCl, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme;

35 (g) incubating the solution in a third solution comprising third solution comprises approximately 1-2 μM, approximately 30 μM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20

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mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 400U/ml of a digestive enzyme;

(h) centrifuging the tissue to obtain isolated cells;

(i) repeatedly resuspending the disassociated cells into a second solution

5 which comprises increasing amounts of  $\text{CaCl}_2$ , Earle's modified salt, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at  
10 approximately 1  $\mu\text{M}$  at a pH of approximately 7.4 to form a solution of isolated cells; and

(j) incubating the isolated cells in a mixture of carbon dioxide and air at approximately 37°C.

15 26. A method of isolating and cultivating rodent myocardial cells comprising,

(a) removing the heart of a rodent;

(b) perfusing the heart with low calcium Tyrode's solution for approximately 3 minutes;

20 (c) perfusing the heart with an enzymatic solution for approximately 8 minutes;

(d) perfusing the heart with a low calcium solution for approximately 3 minutes;

(e) removing the ventricles;

25 (f) mincing the ventricles to isolate myocardial cells;

(g) mixing the cells in a low calcium solution;

(h) resuspending the cells in a solution comprising increasing concentrations of calcium; and

(i) resuspending the cells in culture media solution..

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